Supporting Information

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SI Methods

Analysis of Plasma Renin Activity (PRA) and ACE Activity. PRA and serum ACE activity levels were determined by RIA (RENCTK, DiaSorin; Bühlmann Laboratories). Renin activity was assayed via AngI production. ACE activity was measured using the ACE-REA kit and defined via inhibition by lisinopril. In short, the synthetic substrate [^{3}H]hippuryl-glycyl-glycine was added and cleaved by angiotensin-converting enzyme to [^{3}H]hippuric acid.

Cell Transfer Experiments. In vivo transfer of T cells was performed according to a protocol described in Beyersdorf et al. (1). On day 10 p.i., pan T cells were isolated via MACS (Miltenyi) from the spleens of losartan- or vehicle-treated mice (treatment start on day –3 before immunization). Fifteen mio cells were transferred intraveniously into recipient wild-type mice on day –1 p.i.

1. Beyersdorf N, et al. (2005) Selective targeting of regulatory T cells with CD28 superagonists allow effective therapy of experiental autoimmune encephalomyelitis. *J Exp Med* 202:445–455.

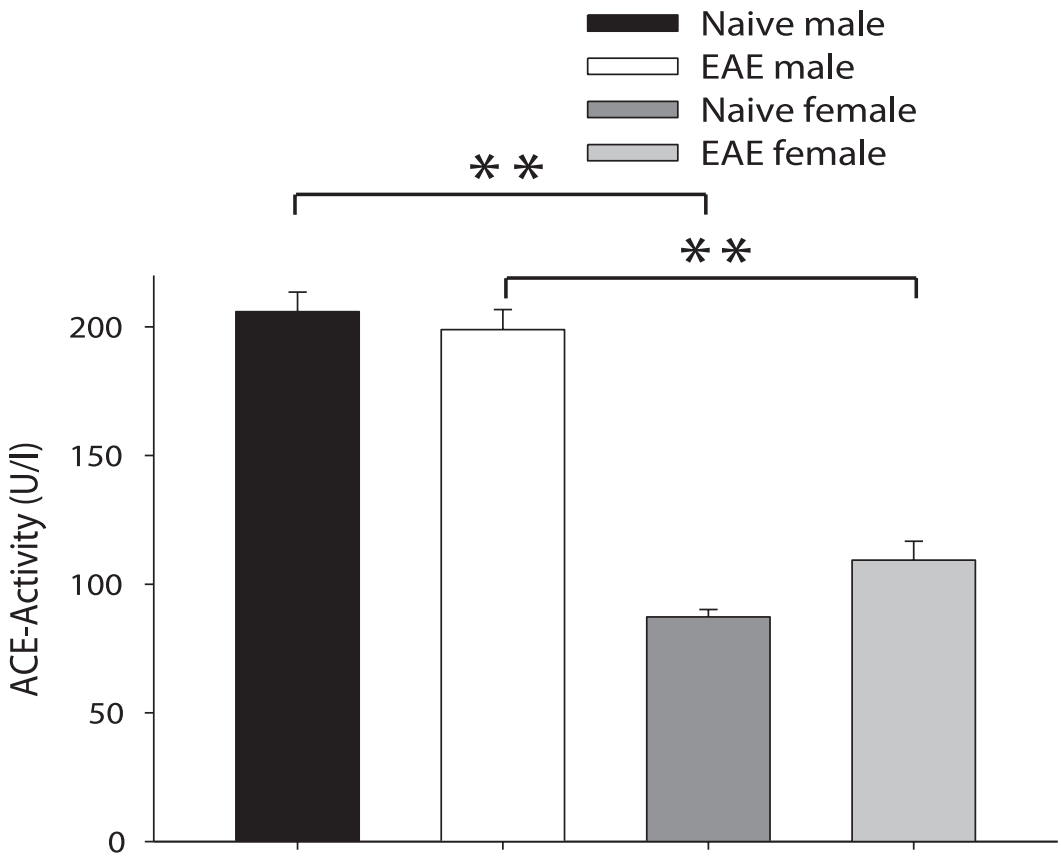

Fig. S1. Expression of plasma ACE in autoimmune inflammation of the CNS. Analysis of serum ACE activities in mice suffering from MOG-EAE (day 31 p.i., n = 6–15 per group) and matched healthy control mice. While the induction of EAE did not influence ACE levels, there was a significant gender effect, with higher ACE activities in male mice (*white and black bars*).

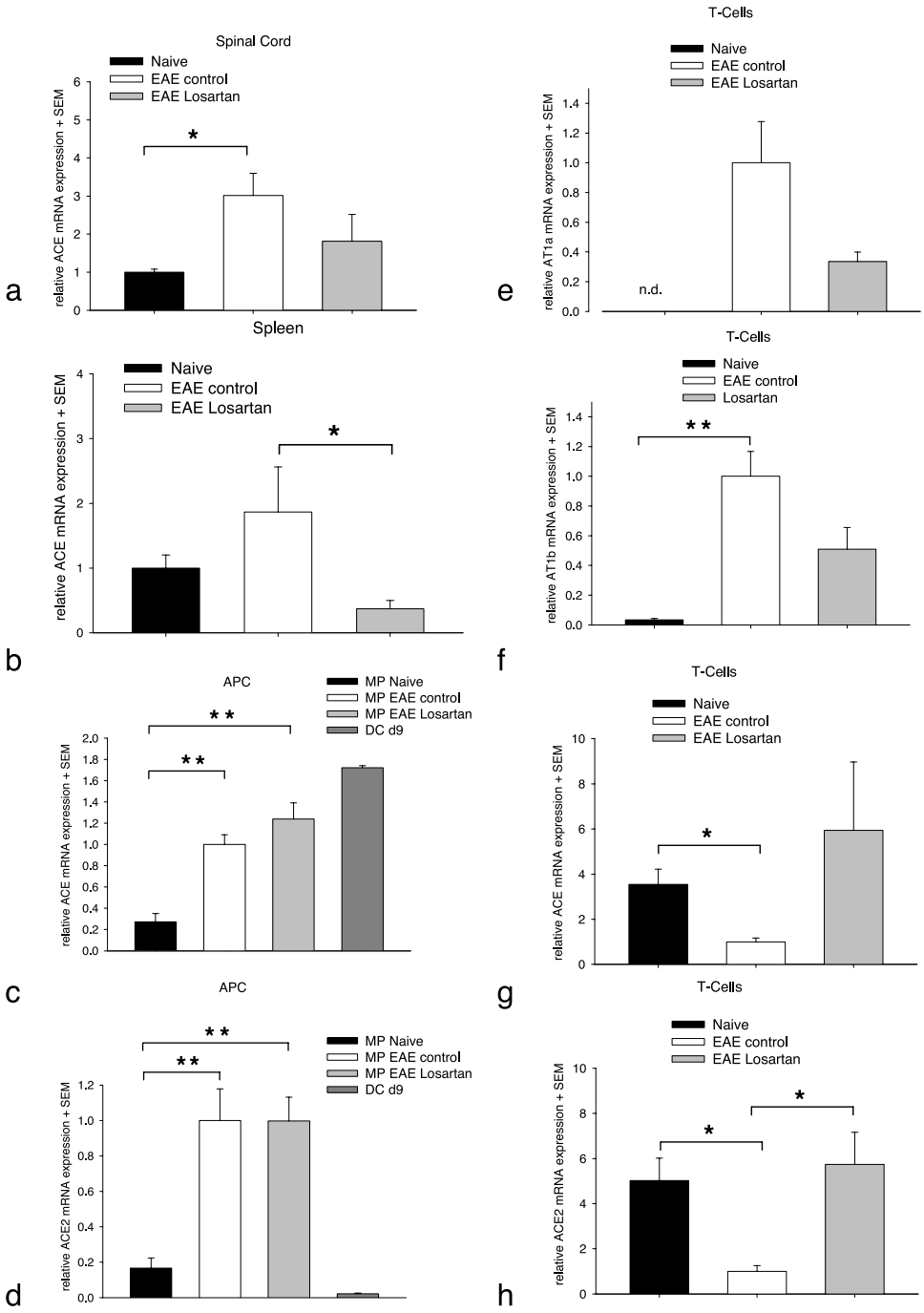

Fig. S2. Expression of RAS components during MOG-EAE (day 31 p.i.) in spinal cord, spleen, T cells, and peritoneal macrophages, as well as in myeloid DCs prepared from bone marrow of naive mice, which were differentiated and matured in vitro for 9 days (d9). Naive control mice (*black bars*) are compared to EAE-diseased mice (*white bars*) and EAE-diseased mice with AT1R blockade via losartan (*gray bars*). Induction of MOG-EAE leads to an (*a*) up to 3-fold increase in ACE expression in the spinal cord, whereas (*b*) losartan treatment reduced ACE expression in the spleen compared to vehicle-treated control mice. In macrophages (MP), (*c*) ACE and (*d*) ACE2 expression are up-regulated after MOG-EAE induction, without modulating effects of losartan treatment. In differentiated myeloid DCs after 9 days in culture (DC d9), ACE and ACE2 expression is also detectable, with a clear preponderance of ACE. The analysis of T cells from MOG-EAE-diseased mice reveals (*e*) an AT1aR expression that is not detected in naive controls and (*f*) an over 10-fold increase in AT1bR expression, while (*g*) ACE expression and (*h*) ACE2 expression in T cells are down-regulated after induction of MOG-EAE. AT1R blockade in MOG-EAE leads to a reduced expression of AT1aR and AT1bR in T cells, while levels of ACE and ACE2 are preserved. Data are presented as relative expression with the respective gene expression in EAE control (*white bars*) set to 1 ($n = 5–6$ per group). n.d. = not detectable.

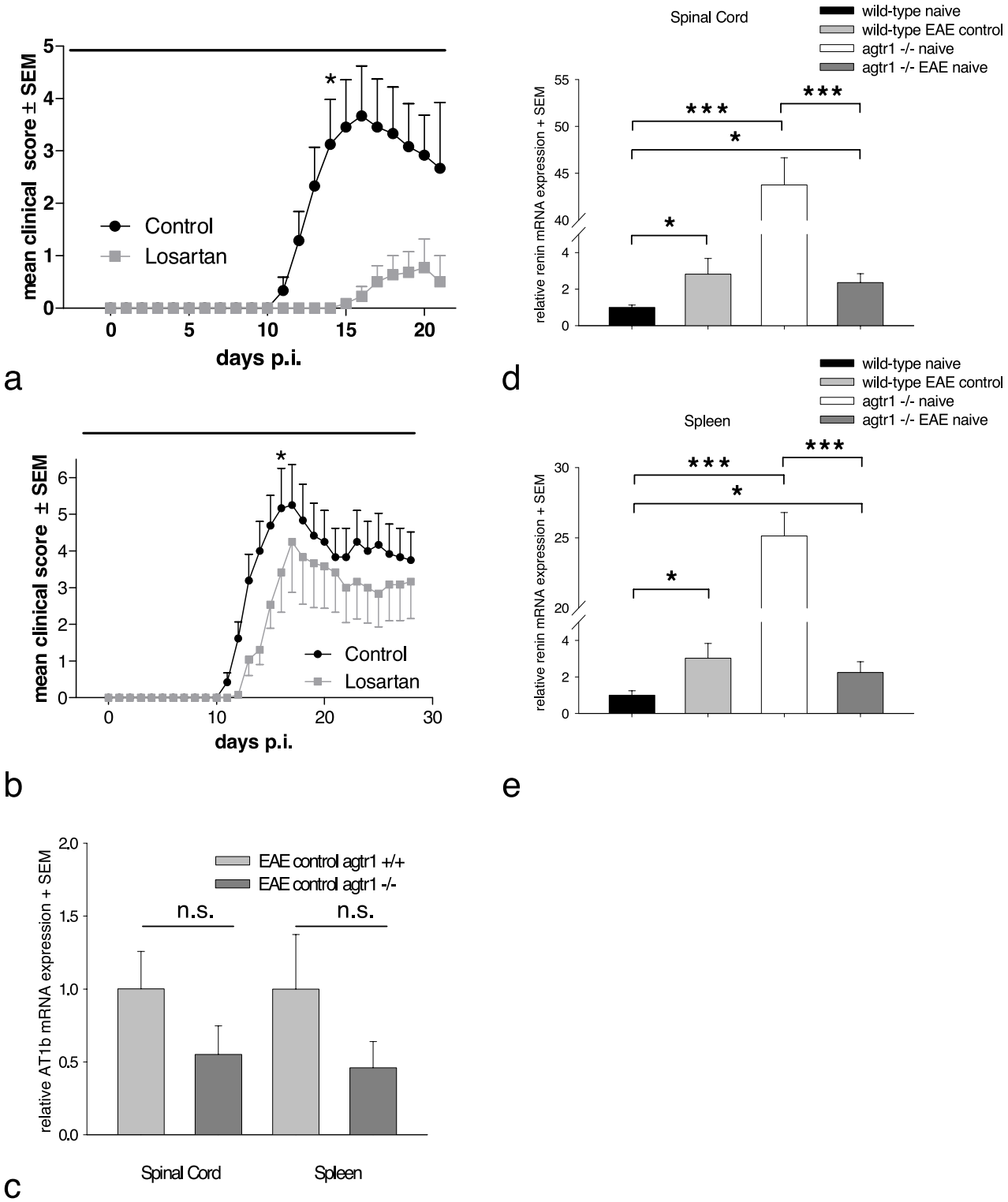

Fig. S3. (*a*) Clinical course of MOG-EAE in female C57BL/6 mice after treatment with losartan. AT1R blockade leads to a significantly ameliorated disease course. Data are pooled from 2 experiments ($n = 12$ vs.11 mice per group, $P < 0.05$ on day 14 p.i.). (*b*) Preventive AT1R blockade with a daily dose of 30 mg/kg body weight losartan (*gray curve*) leads to a later onset of MOG-EAE and an ameliorated later disease phase. Data are pooled from 2 independent experiments ($n = 13$ vs. 16 mice; $P < 0.05$ on day 16 p.i.). Black bar above graphs in (*a*) and (*b*) indicates duration of therapy. (*c*) In AT1aR knockout ($agtr1^{-/-}$) mice, there is no compensatory up-regulation of AT1b receptors in spinal cord and spleen. (*d*) In $agtr1^{-/-}$ mice, renin expression is highly up-regulated in (*d*) spinal cord and (*e*) spleen as compared to wild-type ($agtr1^{+/+}$) mice. In wild-type mice, induction of MOG-EAE increases renin expression in (*d*) spinal cord and (*e*) spleen. Data are presented as relative expression with the respective gene expression in wild-type mice (*black bars*) set to 1 ($n = 4$–7 per group). n.d. = not detectable.

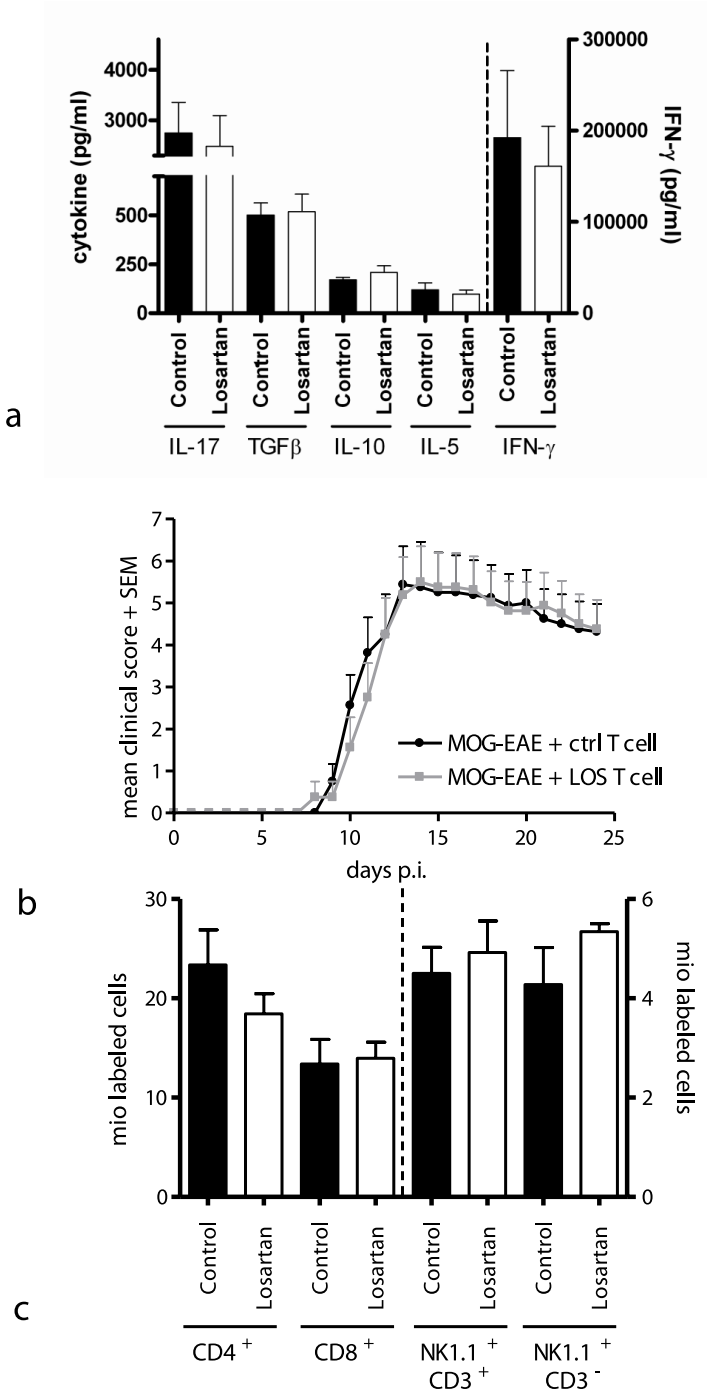

Fig. S4. AT1R blockade does not alter the T-cell response in MOG-EAE. (*a*) Analysis of cytokine production in supernatants from splenocyte primary culture derived from losartan- or vehicle-treated mice. There was no difference in the production of the cytokines IFN-γ, IL-17, or IL-5, as well as IL-10 and TGF-β between losartan- and vehicle-treated mice. Data from a representative experiment with 4 mice per group are shown. (*b*) Clinical course of MOG-EAE after transfer of T cells from mice treated with losartan from day –3 before immunization (''LOS T cell,'' *gray curve*) as compared to vehicle-treated controls (''ctrl T cell,'' *black curve*). Preparation of cells on day 10 p.i. and transfer of 15 mio cells into recipient mice on the day before immunization does not alter their course of MOG-EAE (n = 8 mice per group). (*c*) FACS analyses of T cell and natural killer (NK) cell subsets in the spleen on day 10 of MOG-EAE. In comparison to vehicle application (*black bars*), losartan treatment (*white bars*) does not significantly change numbers of $CD4^+$ or $CD8^+$ T cells, $NK1.1^+$ $CD3^+$ and $NK1.1^+$ $CD3^-$ cells.

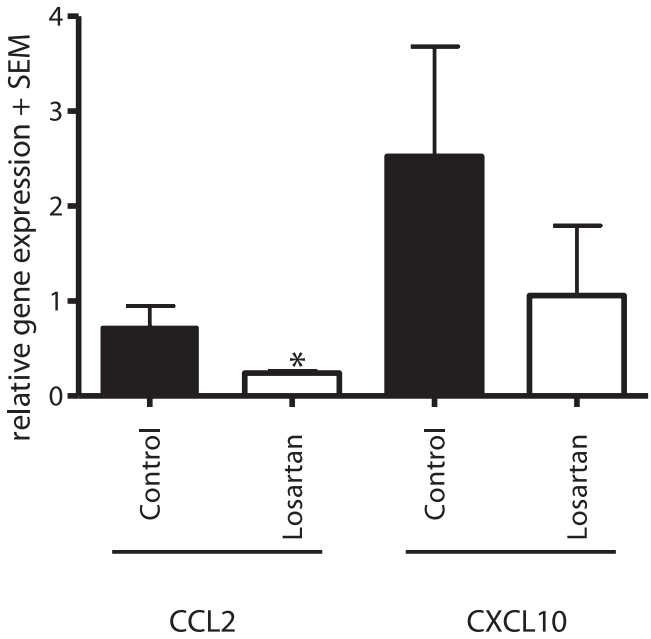

Fig. S5. AT1R blockade impairs chemokine production in macrophages on the mRNA level. Macrophages were prepared on day 16 p.i. of MOG-EAE after in vivo treatment with losartan or vehicle as control, starting 3 days before immunization. In a RT-PCR analysis, there was a significant reduction of CCL2 and a clear trend toward a reduction of CXCL10 after losartan treatment. Data are presented as relative expression with the gene expression in a wild-type mouse set to 1 ($n = 4$ vs. 3 mice per group).